EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 35, No. 5, 431-437, October 2003

NF-κB is involved in the TNF-α induced inhibition of the differentiation of 3T3-L1 cells by reducing PPARγ expression

Gi-Nam Chae[1] and Sahng-June Kwak[1,2]

[1]Department of Biochemistry
College of Medicine, Dankook University
San 29 Anseo-dong, Cheonan 330-714, Korea
[2]Corresponding Author: Tel, 82-41-550-3860;
Fax, 82-41-550-3905; E-mail, ftpn2100@dankook.ac.kr

Accepted 6 October 2003

Abbreviations: IBMX, isobutylmethylxanthine; JNK, c-jun NH_2-terminal kinase; MTT, dimethylthiazol-2-yl diphenyltetrazolium bromide; PPAR, peroxisome proliferator-activated receptor

Abstract

TNF-α, a trimeric cytokine, was known to inhibit differention of preadipocytes to adipocytes. In the present study, we investigated signal mediators working downstream of TNF-α using murine 3T3-L1 cells. TNF-α induced activation of both c-jun NH_2-terminal kinase (JNK) and nuclear transcription factor-κB (NF-κB) in 3T3-L1 cells. Blockage of these two mediators activities by specific inhibitors, SP600125 and Ad-IκBα-SR restored adipogenesis differentiation suggesting their involvement in the inhibited differentiation of 3T3-L1 cells by TNF-α. Consistent with previous studies, peroxisome proliferator-activated receptor gamma (PPARγ) a key transcriptional regulator was remarkably reduced by TNF-α treatment. Compared with adipogenesis, however, SP600125, a chemical JNK inhibitor hardly relieved TNF-α effect on PPARγ expression whereas S32A/S36A mutant of IκBα considerably recovered PPARγ expression, indicating that two signal mediators exploit separable main routes to achieve reduced adipogenesis. These results suggest that inhibition of 3T3-L1 cells differentiation by TNF-α is partly implemented through NF-κB and one of its downstream effectors be PPARγ.

Keywords: adipogenesis; JNK; NF-κB; PPARγ; TNF-α

Introduction

Preadipocyte cell lines, though they have some limitations as in the study of depot-specific differences, contributed enormously to the identification of many biomolecules and the elucidation of transcriptional networks implicated in adipogenesis (Cornelius *et al*., 1994) and many features observed in cell culture system were known to recapitulate well *in vivo*. In the present study, we used 3T3-L1 cells established from mouse fibroblasts (Green and Kehinde 1975; 1976) due to their relative stability of differentiation potential compared to others.

As multipotent or precursor cells differentiate into adipocytes, the characteristic features of this specialized cell type appear including fat-vacuole laden cytoplasm and emergence of marker proteins, which are actually the results from altered gene expression pattern. For this reason, many approaches have been made to explore transcription factors responsible for the differentiation. Among them was found PPARγ, a member of PPAR subgroup of nuclear receptor superfamily that also contains steroid, retinoid, and thyroid hormone receptors (Mangelsdorf *et al*., 1995). Previous studies proved that PPARγ is a transcription factor essential to adipogenesis both *in vivo* and *in vitro* (Tontonoz *et al*., 1994; Hu *et al*., 1995; Rosen *et al*., 1999; Gurnell *et al*., 2000). As a heterodimer with the retinoid receptor RXR, this transcription factor interacts with PPAR responsive element when natural or synthetic ligands bind to the ligand binding domain of PPARγ, thereby modulating expression levels of target genes including adipose tissue fatty acid-binding protein, lipoprotein lipase and phosphoenolpyruvate carboxykinase (Desvergne and Wahli 1999).

TNF-α, a type II membrane protein, was identified as a factor that induced cell death and tumor-related cachexia, also being released as a 17 kDa soluble form by a metalloproteinase at the cell surface. But like all other cytokines, TNF-α is now known to have pleiotropic and pathogenic effects on various tissues and organs, contributing to septic shock, fever, anorexia and even chronic inflammatory diseases (Vassalli 1992). Regarding metabolic point of view, several lines of evidences indicate that this cytokine plays a major role in insulin resistance commonly associated with obesity, and type 2 diabetes (Hotamisligil *et al*., 1993; Uysal *et al*., 1997; Moller 2000) and adipocyte differentiation was profoundly inhibited by TNF-α (Cerami *et al*., 1985; Torti *et al*., 1985).

In this study, our objects were to test whether JNK and NF-κB function as downstream mediators of TNF-α in the inhibition of adipogenesis of 3T3-L1 and to

elucidate whether the down-regulation of PPARγ might be a part of the involved mechanisms, resulting in reduced differentiation caused by activated JNK and NF-κB.

Materials and Methods

Materials

3T3-L1 preadipocyte cell line was purchased from the American Type Culture Collection (Rockville, MD). pGL3-γ1p3000, a reporter containing PPARγ1 genomic promoter (Fajas *et al*., 1997) and adenovirus-IκBα-superrepressor (Ad-IκBα-SR, S32A/S36A mutant of IκBα cloned in adenovirus vector) were kindly provided by Dr. Johan Auwerx (Pasteur Institute, Lille, France) and Dr. Choon-Taek Lee (Seoul National University, Korea) respectively while pcDNA3 was obtained from Invitrogen (San Diego, CA), Passive Lysis 5X Buffer form Promega Corp. (Madison, WI) and D-luciferin from PharMigen (San Diego, CA). Phospho-plus-JNK (Thr183/Tyr185) antibody kit and Phospho-c-Jun (ser63) antibody were provided by Cell Signalling Technology (Beverly, MA), IκBα antibody and actin antibody by Santa Cruz Biotechnology Inc. (Santa Cruz, CA), TNF-α by R&D System (Minneapolis, MN), and SP600125 by Alexis Biochemicals (San Diego, CA).

Cell culture and adipocyte differentiation

3T3-L1 cells were passaged in L1 medium (DMEM plus 10% FBS, 100 U/ml penicillin and 100 g/ml streptomycin) housed in a humidified incubator set at 5% CO_2 and 37°C. To induce adipogenesis, 3T3-L1 cells ($8\times10^4/cm^2$) were plated into culture vessels (24 well plate for luciferase assay, 6 well plate for Oil Red O staining, and 60 mm dish for immunoblotting) and maintained 2 days (in L1 medium) after reaching confluence. Then, the cells were subjected to differentiation medium (DMEM containing 10% FBS, 0.5 mM isobutylmethylxanthine (IBMX), 1 M dexamethasone and 10 g/ml insulin) for 2 days. Finally, differentiation medium was replaced with adipocyte growth medium (DMEM plus 10% FBS and 10 g/ml insulin) that was refreshed every 2 days. Cytotoxcicity of TNF-α ranging from 0.1 to 10 ng/ml was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) test at day 3 and day 7 after the incubation with this cytokine. To inhibit adipogenesis, TNF-α (final concentration 5 ng/ml) was treated 30 min prior to the addition of IBMX, dexamethasone and insulin. To block JNK and NF-κB activities, SP600125 (final concentration 20 M) and Ad-IκBα-SR (5 multiplicity of infection) were given respectively 1 h before TNF-α treatment. Denoted time points of cell harvest were from the start of incubation with differentiation medium.

Quantification of lipid accumulation

At day 7 after differentiation medium was added, cells were retrieved and neutral lipid accumulation was measured using a previously published method (Sen *et al*., 2001). Briefly, cells were washed with PBS, fixed with 10% buffered formalin and stained with Oil Red O solution (0.5 g in 100 ml isopropanol) for 10 min. After the staining solution was removed, the dye retained in the cells was eluted into isopropanol and OD_{540} was determined.

SDS-PAGE and immunoblotting

Harvested cells were lysed with 50 mM Tris (pH 7.4) containing 1% Nonidet P-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4 and Complete™ protein inhibitor (Boehringer Mannheim). Lysates were centrifuged at 14,000 rpm for 30 min at 4°C and supernatants were collected. Protein concentrations were determined using BCA reagents (Pierce). After boiled with 3 X loading buffer, equal amounts (30 g protein per lane) were electrophoresed in 12% SDS-PAGE gels, which were blotted onto polyvinylidene difluoride membranes. These blots were blocked with Tris-buffered saline with 0.1% Tween 20 (TBS-T) and 5% non-fat dried milk. The membranes were incubated with antibodies (1:1000 dilution, in TBS-T plus 5% non-fat dried milk) specific for phospho-JNK, JNK, phospho-c-Jun, IκBα or actin respectively for 1 h at room temperature, then washed with TBS-T and reacted with anti-rabbit antibody conjugated with horseradish peroxidase. Having washed away unbound anti-rabbit antibody, antigen-antibody complexes were visualized by chemiluminescence.

Reverse transcription PCR (RT-PCR)

3T3-L1 cells were cultured in 60 mm dishes and treated with TNF-α, SP600125, and Ad-IκBα-SR respectively. 5 dishes were prepared for only adipogenesis; 4 dishes, pretreated with TNF-α and differentiated; another 4 dishes, pretreated with Ad-IκBα-SR and TNF-α, then differentiated; last 4 dishes, pretreated with SP600125 and TNF-α, then differentiated. Cells were harvested at the time points indicated (Figure 5).

Total RNA was isolated with Trizol reagent (Gibco-BRL) according to the manufacturer's protocol. For each sample, reverse transcription (RT) was performed with 2 g total RNA and resultant cDNA population was amplified by PCR for PPARγ that was compared with concurrently measured β-actin expression level. PCR primers were as follows: 5'CCTCTCCGTGATG-

GAAGACC3' (sense) and 5'GCATTGTGAGCATCCC-CAC3' (antisense) were for PPAR whereas 5'GTGG-GGCGCCCCAGGCACCA3' (sense) and 5'CTCCTTA-ATGTCACGCACGATTTC3' (antisense) for β-actin.

Transfection and luciferase assay

3T3-L1 cells (5×10^5) were plated into 60 mm dish and on the next day transfected with pGL3-γ1p3000 and pcDNA3 (3 g in total, 4:1 ratio) using Lipo-fectAMINE PLUS (Invitrogen). When 80% confluent, transfected cells were split into 6 dishes, which were placed under G418 selection (400 g/ml). Each 6 dishes were duplicated when subcultured cells became 80% confluent. For each mixed population that survived G418 selection, one part was saved and the other was tested as follows: Cells from each selected batch were plated into 2 wells of a 24 well plate (5×10^4/well) and cultured in L1 medium, chang-ed every 2 days. At 48 h after the confluence, one well (representing signal) was changed with differenti-ation medium whereas the other well (representing noise) was still maintained in L1 medium. At 60 h posterior to the addition of differentiation medium, cells were washed with PBS and lysed with Passive Lysis Buffer (25 mM Tris-phosphate pH 7.6, 2 mM DTT, 2 mM 1,2-diaminocyclohexane-N,N,N',N'-tetra-acetic acid, 10% glycerol, 1% Triton X-100). After protein concentrations were determined with BCA Reagents, 20 g protein was assayed with luciferase reaction mixture (25 mM glycylglycine, 15 mM MgSO4, 1 mg/ml BSA, 5 mM ATP, and 1 mM D-luciferin). Among 6 mixed populations, was selected a batch that showed the highest signal/noise ratio and named as 3T3-L1/PPARγ cells. These 3T3-L1/PPARγ cells were expanded to 5th passage, which were used in all the luciferase assays done henceforth. For one set of experiment, 3T3-L1/PPARγ cells (5×10^4/well) were plated into 8 wells of 24 well plate and 1st well was maintained only with L1 medium as a negative con-trol; 2nd well, differentiated with differentiation medi-um; 3rd well, pretreated with Ad-IκBα-SR and differ-entiated; 4th well, pretreated with SP600125 and differentiated; 5th well, pretreated with TNF-α and differentiated; 6th well, pretreated with Ad-IκBα-SR and TNF-α in sequence, then differentiated; 7th well pretreated with SP600125 and TNF-α in order and differentiated; 8th well pretreated with Ad-IκBα-SR, SP600125, and TNF-α sequentially, then differentiated whereas 3T3-L1 cells were seeded and differentiated in 9th well. All the cell culture conditions and drug treatment protocols used here were the same as des-cribed above (cell culture and adipocyte differentiation in Materials and Methods) except that cells were harvested 60 h after the addition of differentiation medium for luciferase assays.

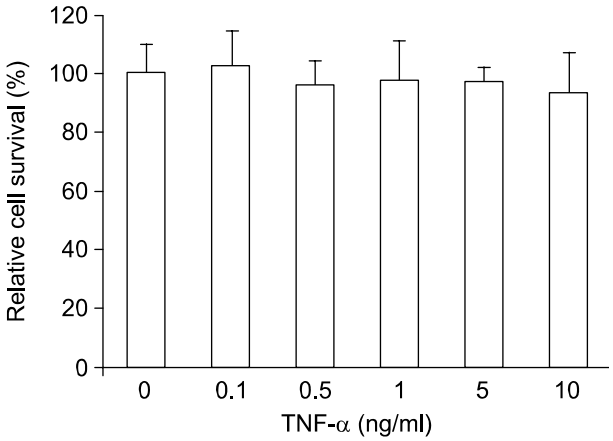

Figure 1. Cytotoxicity of TNF-α on 3T3-L1 cells. 3T3-L1 cells were subjected to adipogenesis protocol as described in Materials and Methods, also being treated with TNF-α ranging from 0.1 to 10 ng/ml, when initially confluent. At day 3 and day 7 after TNF-α treatment, the amounts of viable cells were measured by MTT test and presented as the percentages of untreated viable cells. Cell survival patterns of day 3 and day 7 were the same. Shown above is the result from day 3 experiment. The data represent the mean±SE of three inde-pendent experiments, each performed in duplicate.

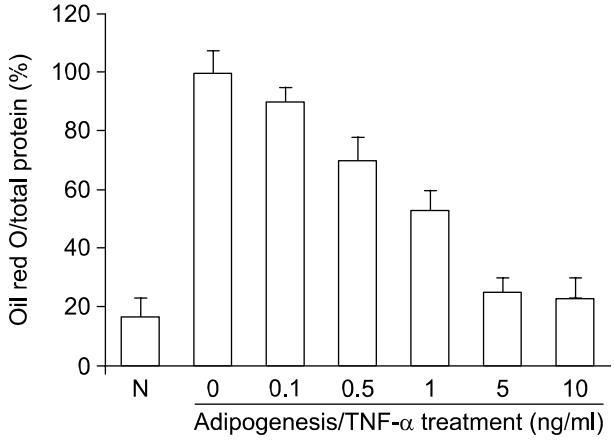

Figure 2. TNF-α induced inhibition of the adipogenesis of 3T3-L1 cells. 3T3-L1 cells, when confluent, were treated with TNF-α ranging from 0.1 to 10 ng/ml and incubated with differentiation medium for 2 days, which was replaced with adipocyte growth medium. Neutral lipid accumulation, quantified by Oil Red O dye at day 7, was used as an index of adipogenesis of 3T3-L1 cells. Each bar is expressed as the percentage compared with the neutral lipid accumulated without TNF-α treatment. Bar marked N represents undifferentiated 3T3-L1 cells that were maintained with L1 medium for the same period. Reduced lipid synthesis was dose-dependent and saturation effect was observed from 5 ng/ml on. The values are the mean±SE of three independent experiments.

Results

Effects of TNF-α on adipogenesis

We performed MTT test to exclude the possibility of cytotoxicity of TNF-α that could misinterpretate cell death or retarded cell proliferation as inhibited adipogenesis. As shown in Figure 1 (day 3 data presented), noticeable differences in cell death or inhibited cell proliferation were not observed up to 10 g/ml of TNF-α when measured at both day 3 and day 7 after TNF-α treatment while dose-dependent inhibition of adipogenesis clearly appeared, reaching the saturation level near 5 g/ml (Figure 2).

In 3T3-L1 cells, phosphorylation of JNK and IκBα protein levels were monitored in response to TNF-α stimulation (5 g/ml). Though small portion of JNK was already phosphorylated, more of it became phosphorylated after the addition of TNF-α (Figure 3A) and 20 M of SP600125 was enough to prevent c-Jun phosphorylation by JNK (Figure 3B). Following TNF-α treatment, IκBα was also effectively degraded (Figure 3C), releasing free NF-κB that could reach its target genes.

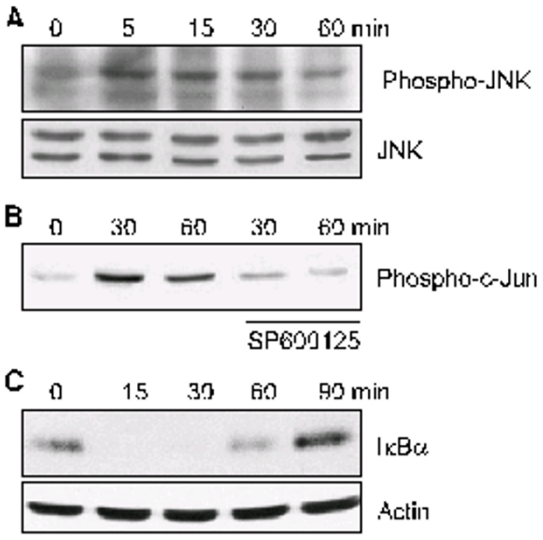

Figure 3. Activation of JNK and NF-κB in 3T3-L1 cells by TNF-α treatment. 3 different sets of 3T3-L1 cells were prepared respectively as shown and pretreated with TNF-α (5ng/ml), and underwent adipogenesis. Cells were harvested at the denoted time points past the addition of differentiation media. Cell lysates were made and centrifuged, yielding supernatants that were processed for SDS-PAGE. In panel A, the membrane was initially probed with JNK antibody that was stripped off later. Then washed membrane was immunoblotted with phospho-JNK antibody. In panel B, the membrane was reacted with phospho-c-Jun antibody. Cells for last two lanes were pretreated with a JNK inhibitor, SP600125 (20 M) prior to TNF-α whereas first three lanes were treated only with TNF-α. In panel 3, the membrane was initially immunoblotted with IkBα antibody and reprobed with actin antibody. All experiments were independently repeated twice, resulting in reproducible patterns, of which were shown representative ones.

Differentiated 3T3 cells contained more than 5 times extractable lipids compared with the undifferentiated (Figure 4, bars marked +, -). Ad-IκBα-SR and SP-600125 by themselves affected the adipogenesis of 3T3-L1 insignificantly (Figure 4, bars marked SR, SP). Inhibition signal from TNF-α was substantially alleviated by Ad-IκBα-SR (Figure 4, bar marked SR/TNF-α) and also became diminished slightly less by SP600125 (Figure 4, bar marked SP/TNF-α). Certain synergism between Ad-IκBα-SR and SP600125 was surmised (Figure 4, bar marked SR+SP/TNF-α).

Effect of TNF-α on expression of PPARγ

Expression levels of PPARγ were estimated by semi-quantitative RT-PCR at denoted time points past the addition of differentiation medium. PPARγ appeared at day 1 and increased till day 4 (Figure 5A) and became hardly detectable when TNF-α was pretreated (Figure 5B). This inhibition of PPARγ expression was notably recovered by IκBα-SR (Figure 5C) while the effect of SP600125 was weak (Figure 5D). Undif-

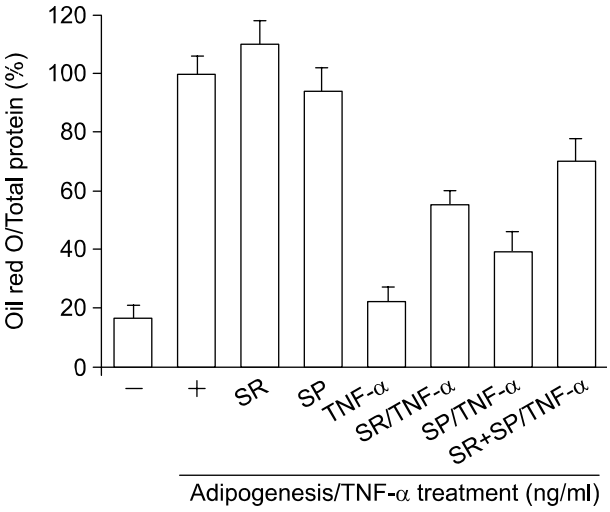

Figure 4. Altered adipogenesis by modulation of JNK and NF-κB activities. 3T3-L1 cells were treated as followed. For bar marked -, cells were maintained with L1 medium for the same period; for bar marked +, subjected to adipogenesis protocol (confluent state 2 days, differentiation medium 2 days and adipocyte growth medium 5 days in order); for bar marked SR, pretreated with Ad-IκBα-SR and subjected to adipogenesis protocol; for bar marked SP, pretreated with SP600125 and subjected to adipogenesis protocol; for bar marked TNF-α, pretreated with TNF-α and subjected to adipogenesis protocol; for bar marked SR/TNF-α, pretreated with Ad-IκBα-SR and TNF-α then subjected to adipogenesis protocol; for bar marked SP/TNF-α, pretreated with SP600125 and TNF-α then subjected to adipogenesis protocol; for bar marked with SR+SP/TNF-α, pretreated with Ad-IκBα-SR, SP600125 and TNF-α then subjected to adipogenesis protocol. Neutral lipid was measured as in Figure 2 at day 7 after differentiation media were added. The values are the mean±SE of three independent experiments.

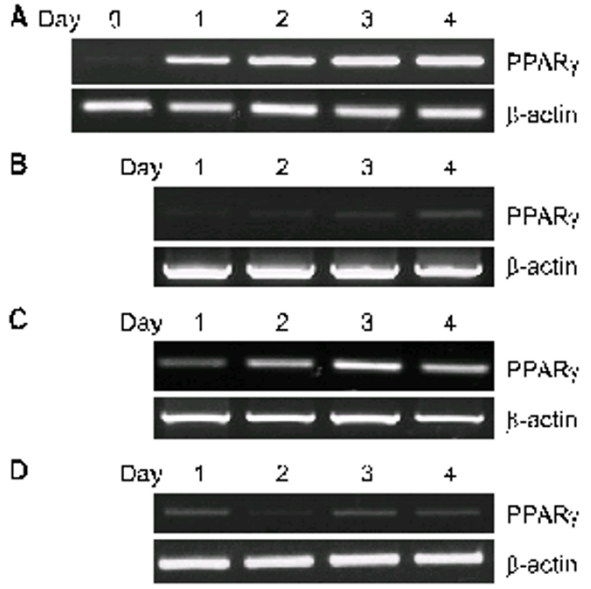

Figure 5. Expression levels of PPARγ influenced by TNF-α treatment PPARγ expression levels were assessed by RT-PCR as described in Materials and Methods. In panel A, 3T3-L1 cells underwent adipogenesis; in panel B, pretreated with TNF-a and underwent adipogenesis; in panel C, pretreated with Ad-IkBa-SR, TNF-α and underwent adipogenesis; in panel D, pretreated with SP600125, TNF-α and underwent adipogenesis. All experiments were independently repeated three times, resulting in reproducible patterns, of which were shown representative ones.

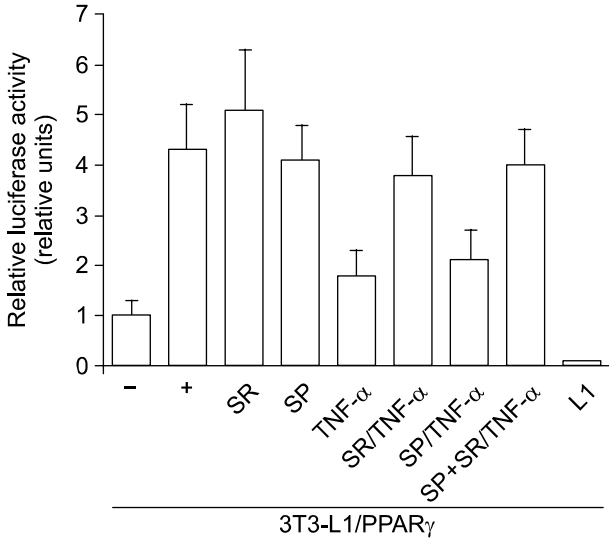

Figure 6. Altered PPARγ promoter activity by modulation of JNK and NF-κB signals. Promoter activity of PPARγ was measured by luciferase assay. 3T3-L1/PPARγ cells were prepared and treated as described in in Materials and Methods. Briefly, 3T3-L1/PPARγ cells were just maintained in L1 medium for bar marked -; underwent adipogenesis for bar marked +; pretreated with Ad-IκBα-SR and underwent adipogenesis for bar marked SR; pretreated with SP600125 and underwent adipogenesis for bar marked SP; pretreated with TNF-α and underwent adipogenesis for bar marked TNF-α; pretreated with Ad-IκBα-SR and TNF-α then underwent adipogenesis for bar marked SR/TNF-α; pretreated with SP600125 and TNF-α then underwent adipogenesis for bar marked SP/TNF-α; pretreated with Ad-IκBα-SR, SP600125 and TNF-α then underwent adipogenesis for bar marked with SR+SP/TNF-α. The data represent the mean±SE of three independent experiments, each performed in triplicate.

ferentiated 3T3-L1/PPARγ cells (Figure 6, bar marked -) showed some basal luciferase activity compared with differentiated 3T3-L1 cells (Figure 6, bar marked L1), however once differentiated, more than 4 fold increment of luciferase activity was observed (Figure 6, bar marked +), ensuring reliable signal to basal difference. TNF-α treatment reduced luciferase activity by half (Figure 6, bar marked TNF-α) and IkBa-SR counteracted TNF-α activity to some degree (Figure 6, bar marked SR/TNF-α), whereas the effect of SP600125 was almost negligible (Figure 6, bar marked SP/TNF-α). Opposed to neutral lipid accumulation, synergism for recovering luciferase activity between Ad-IκBα-SR and SP600125 was barely seen (Figure 6, bar marked SR+SP/TNF-α).

Discussion

Having formed trimeric complex, TNF-α interacts with its two different receptors termed TNFR1 and TNFR2, which, in response, oligomerize and become activated, relaying extracellular information inside. Depending upon cellular context, TNF-α has been shown to initiate a variety of responses and many studies in non-fat tissues to date showed that NF-κB and JNK play main roles as downstream molecular components of TNF-α in involved signaling pathways (Heller and Kronke 1994; Baker and Reddy 1998; Orlinick and Chao 1998).

Though in 3T3-L1 cells TNF-α induced signals are delivered inside *via* TNFR1 that interacts more readily with Fas-associated death domain than TNFR2 (Baker and Reddy 1998; Xu *et al*., 1999), cell death rarely occurred within TNF-α concentration used in our work (Figure 1), meaning that reduced lipid accumulation largely resulted from inhibited adipogenesis (Figure 2). Since constitutively increased JNK activity was reported in certain batches of undifferentiated 3T3-L1 cells (Ryden *et al*., 2002), our cells were evaluated for its basal activity and responsiveness to TNF-α, resulting that JNK in our batch still could be activated (Figure 3A, B, C). Though phosphorylation of serine residue in A/B domain of PPARγ by mitogen-activated protein kinases (MAPK) was reported to decrease transcriptional activity of this receptor (Adams *et al*., 1997), overall effects of these MAPK in adipogenesis are controversial, either in favor of or against adipogenesis (Kim *et al*., 2001; Klemm *et al*., 2001; Prusty

et al., 2002). Under our experimental setting, suppression of JNK enhanced adipogenesis slightly (Figure 4, bar marked SP/TNF-α). Consistent with previous studies (Zhang *et al*., 1996; Xing *et al*., 1997), TNF-α profoundly inhibited both adipogenesis (Figure 4, bar marked TNF-α) and PPARγ expression (Figure 5B). When NF-κB was blocked by Ad-IκBα-SR, adipogenesis was substantially recovered (Figure 4, bar marked SR/TNF-α) with increased PPARγ expression and luciferase activity (Figure 5C; Figure 6, bar marked SR/TNF-α) while JNK inhibitor affected PPARγ expression little. In conclusion, though direct mediators on PPARγ gene were not defined, our data strongly suggested that inhibition of differentiation of 3T3-L1 cells by TNF-α be partly implemented through NF-κB and one of its downstream effectors be PPARγ.

Acknowledgement

The present research was conducted by the research fund of Dankook University in 2001.

References

Adams M, Reginato MJ, Shao D, Lazar MA, Chatterjee VK. Transcriptional activation by peroxisome proliferator-activated receptor gamma is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. J Biol Chem 1997;272:5128-32

Baker SJ, Reddy EP. Modulation of life and death by the TNF receptor superfamily. Oncogene 1998;17:3261-70

Cerami A, Ikeda Y, Le Trang N, Hotez PJ, Beutler B. Weight loss associated with an endotoxin-induced mediator from peritoneal macrophages: the role of cachectin (tumor necrosis factor). Immunol Lett 1985;11:173-7

Cornelius P, MacDougald OA, Lane MD. Regulation of adipocyte development. Annu Rev Nutr 1994;14:99-129

Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 1999;20: 649-88

Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J. The organization, promoter analysis, and expression of the human PPARγ gene. J Biol Chem 1997;272:18779-89

Green H, Kehinde O. An established preadipose cell line and its differentiation in culture. II. Factors affecting the adipose conversion. Cell 1975;5:19-27

Green H, Kehinde O. Spontaneous heritable changes leading to increased adipose conversion in 3T3 cells. Cell 1976; 7:105-13

Gurnell M, Wentworth JM, Agostini M, Adams M, Collingwood TN, Provenzano C, Browne PO, Rajanayagam O, Burris TP, Schwabe JW, Lazar MA, Chatterjee VK. A dominant-negative peroxisome proliferator-activated receptor gamma (PPARγ) mutant is a constitutive repressor and inhibits PPARγ-mediated adipogenesis. J Biol Chem 2000;275:5754-9

Heller RA, Kronke M. Tumor necrosis factor receptor-mediated signaling pathways. J Cell Biol 1994;126:5-9

Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 1993;259:87-91

Hu E, Tontonoz P, Spiegelman BM. Transdifferentiation of myoblasts by the adipogenic transcription factors PPARγ and C/EBP α. Proc Natl Acad Sci USA 1995;92:9856-60

Kim SW, Muise AM, Lyons PJ, Ro HS. Regulation of adipogenesis by a transcriptional repressor that modulates MAPK activation. J Biol Chem 2001;276:10199-206

Klemm DJ, Leitner JW, Watson P, Nesterova A, Reusch JE, Goalstone ML, Draznin B. Insulin-induced adipocyte differentiation. Activation of CREB rescues adipogenesis from the arrest caused by inhibition of prenylation. J Biol Chem 2001; 276:28430-5

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P. The nuclear receptor superfamily: the second decade. Cell 1995;83:835-9

Moller DE. Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes. Trends Endocrinol Metab 2000;11:212-7

Orlinick JR, Chao MV. TNF-related ligands and their receptors. Cell Signal 1998;10:543-51

Prusty D, Park BH, Davis KE, Farmer SR. Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor gamma (PPARγ) and C/EBPalpha gene expression during the differentiation of 3T3-L1 preadipocytes. J Biol Chem 2002;277:46226-32

Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM. PPARγ is required for the differentiation of adipose tissue *in vivo* and *in vitro*. Mol Cell 1999;4:611-7

Ryden M, Dicker A, van Harmelen V, Hauner H, Brunnberg M, Perbeck L, Lonnqvist F, Arner P. Mapping of early signaling events in tumor necrosis factor-α-mediated lipolysis in human fat cells. J Biol Chem 2002;277:1085-91

Sen A, Lea-Currie YR, Sujkowska D, Franklin DM, Wilkison WO, Halvorsen YD, Gimble JM. Adipogenic potential of human adipose derived stromal cells from multiple donors is heterogeneous. J Cell Biochem 2001;81:312-9

Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPARγ 2, a lipid-activated transcription factor. Cell 1994;79:1147-56

Torti FM, Dieckmann B, Beutler B, Cerami A, Ringold GM. A macrophage factor inhibits adipocyte gene expression: an *in vitro* model of cachexia. Science 1985;229:867-9

Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-α function. Nature 1997;389:610-4

Vassalli P. The pathophysiology of tumor necrosis factors. Annu Rev Immunol 1992;10:411-52

Xing H, Northrop JP, Grove JR, Kilpatrick KE, Su JL, Ringold GM. TNF alpha-mediated inhibition and reversal of adipocyte differentiation is accompanied by suppressed expression of PPARgamma without effects on Pref-1 expression. Endocrinology 1997;138:2776-83

Xu H, Sethi JK, Hotamisligil GS. Transmembrane tumor necrosis factor (TNF)-α inhibits adipocyte differentiation by selectively activating TNF receptor 1. J Biol Chem 1999;274: 26287-95

Zhang B, Berger J, Hu E, Szalkowski D, White-Carrington S, Spiegelman BM, Moller DE. Negative regulation of peroxisome proliferator-activated receptor-gamma gene expression contributes to the antiadipogenic effects of tumor necrosis factor-α. Mol Endocrinol 1996;10:1457-66